IMMUNITY IN POLIOMYELITIS[1,2]

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Immunity in poliomyelitis is a particularly timely topic in view of recent studies from many laboratories. The theme of immunology presented here, however, will be launched from a series of events occurring on the small Pacific island of Guam, and subsequently subjected to laboratory and armchair analysis.

No attempt will be made to review much of the older literature on the immunity of poliomyelitis. As an excuse, a cue will be taken from Dr. Sabin, who said at the end of a Bela Schick Lecture (1), "I have drawn largely on my own studies, and may have left the impression that very little other work has been done on the subject that is worth mentioning". Then he quoted Professor Szent-Györgi who, when challenged at the end of a lecture by another scientist for not having mentioned his work, replied: "If I had taken time to describe his many contributions, I should have had no time left to tell of my own."

Articles about poliomyelitis are characterized by two common faults of popular medical subjects: excessive quantity and frequent lack of quality. The tremendous amount of trash cluttering up the literature renders all polio work most difficult. Rather often work of poor quality casts doubt on sound conclusions based on perfectly good work, but to remove the doubt it must be repeated, some times not just once but several times. The thousands of published articles nevertheless do include scores of reports of excellent work. In fact, the writings of Caverly, Flexner, Landsteiner, Wickman, Frost, Aycock, Trask, and many others provide precedent for practically every idea and every experiment reported since. Amateur scientists, however, many seeking easy publicity, have written profusely on poliomyelitis on the slightest scientific pretext.

Before launching into the subject of immunity in poliomyelitis, certain concepts should be clarified. First, infection with the virus of poliomyelitis must be clearly distinguished from the recognized paralytic or nonparalytic disease. Practically everyone eventually, and frequently very early in life, becomes infected with the several viruses, which we call poliomyelitis viruses, but the clinical syndrome called poliomyelitis is a rare manifestation, perhaps only a "complication" of these infections. Second, it is quite possible that the factor of resistance which prevents the majority of persons from manifesting central nervous system disease or "complication" during infection is *not immunity*, for in poliomyelitis this non-specific factor of resistance presumably is ordinarily present at the time

[1] Presidential Address read in part at the 49th Meeting of the Society of American Bacteriologists, Cincinnati, Ohio on May 18, 1949.

[2] The original work reported was aided by a grant from the National Foundation for Infantile Paralysis, Inc.

The invaluable assistance of Gladys E. Sather, M. P. H., and E. C. Roberts, Ph.D. is acknowledged. In the future publications of the completed studies summarized in this address, either or both will be coauthors.

of the first experience with the virus. True, specific immunity, must be the result of previous infection. Its functions are not well determined, for it may either prevent reinfection or modify future infection and thus render the likelihood of disease (already quite unlikely because of the natural resistance) still more unlikely. To the epidemiologist, the role of immunity in preventing reinfection and the carrier state is by far the more important possible function.

For several decades, statistical epidemiological studies have been made on observed or reported cases of poliomyelitis and such studies are multiplying at a great rate. However, conclusions drawn from these studies are based on a whole series of unproved suppositions—some logical, some rather dubious. It is presumed, for instance, that diagnosis has been accurate and that cases have been uniformly reported from all areas and at all times. Some concepts of immunity in poliomyelitis are derived from such studies, and others are based on still other assumptions. Some previously held concepts will be challenged and an attempt will be made to substitute or suggest alternate hypotheses for some of the present weak ones.

The first question I would like to raise is whether a single infection, clinical or subclinical, produces a lifelong or long-enduring immunity, such as that which occurs in measles. The second is closely related and can scarcely be separated from it: does poliomyelitis immunity prevent reinfection and the carrier state completely, again as we believe occurs in measles, or does it simply modify the clinical aspects of infection?

Because the age distribution of patients with clinical poliomyelitis had been observed to be roughly similar to that of patients with measles and chicken pox, it was postulated and generally accepted many years ago that immunity resulted from a single infection, apparent or inapparent, and that this immunity endured for life. Poliomyelitis has been listed in practically every textbook as one of the "immunizing infections of childhood". Acquired immunity appeared to satisfactorily explain the rareness of cases among older children and adults, an explanation accepted in the case of most acute communicable diseases of childhood. Proof of this is available, however, only in those diseases for which two characteristics may be demonstrated: (*a*) that adults are known to be just as susceptible as children if not previously exposed, otherwise immunity cannot be assumed to be responsible for a lower incidence among adults; (*b*) that persons having once had the disease must be observed to remain immune after many years' residence in an isolated environment where re-exposure and stimulation of immunity cannot occur, otherwise repeated, frequent exposure might account for apparent immunity. This proof is available in the case of measles, mumps and chicken pox, for there are reliable reports of outbreaks of these diseases in relatively isolated "virgin" populations, in which all age groups were equally involved. Several outbreaks of what has been *called* poliomyelitis have been reported in "virgin" populations (2–5). These also have affected both adults and children. Most of these were on tropical, Western Pacific islands. However, these reports must be considered with reservations because the infecting virus has not been isolated or identified, hence the disease could have been Japanese B encephalitis or one of

the many other infections of the central nervous system. We have recently demonstrated antibodies to Japanese B or other related viruses in sera from natives from some of these islands (6, 7). Most students of poliomyelitis today view these reports with considerable doubt about the validity of the etiological diagnosis (3). A more recent outbreak on the Nicobar Islands off the coast of India, referred to by Pandit, was the subject of laboratory investigation but the results are not yet known (8).

An opportunity has been presented recently in a series of visits to the island of Guam to observe or obtain information regarding an interesting series of outbreaks which stimulate thought on this problem. Mumps had not occurred in some parts of the island for 18 years, and any place on the island for possibly 8 years. American dependants probably introduced the virus some time in 1947. Practically every native not previously known to have had the disease, including adults, became infected, according to clinical or serological observations, the latter made in our laboratory. Those previously infected were immune. At the same time there occurred an outbreak of Japanese B encephalitis (6, 7, 9) which might have been reported eventually as poliomyelitis, as was an outbreak in 1899 (10), had there not been alert Navy doctors present. Unless consultants had been called, it might even then have gone on record as an epidemic of mumps meningoencephalitis. Next, measles came to Guam after an absence of 15 years, and similar observations to those of mumps were made. Children and adults alike developed the disease unless they had had it previously.

The next incident to occur on Guam, I consider highly significant. Poliomyelitis appeared about 6 months ago, but in this instance was manifest almost entirely among American children and adults, *not* among Guamanians as were measles and mumps. Finally, only four possible cases of poliomyelitis were found among the natives (11, 12). Yet, Captain C. K. Youngkin, Navy Medical Officer and Director of the Department of Public Health on Guam has told me that prior to this outbreak he has never been able to find any evidence of paralytic poliomyelitis among Guamanians although he has looked for it carefully. Before the outbreak we had tested serum from the native children for neutralizing antibodies to the Lansing strain of the poliomyelitis virus and found them present in children much younger than in the United States. Every serum of the small group then tested, beginning with those of 1 year of age, through 4 years, was positive. These same sera have been tested for antistreptolysin "O", and high titers of this antibody appear at a much earlier age than in sera collected in the United States. These laboratory studies will be discussed in more detail later.

Diphtheria infection, too, is present on Guam though seldom recognized clinically. Jacobziner (13), through the use of the Schick test, found that very few children even at 1 to 3 years of age are Schick positive (susceptible) yet the disease was not recognized and no artificial immunization had been practiced.

In contrast then to measles and mumps, poliomyelitis, diphtheria and beta hemolytic streptococcal infections, though attacking the population at an earlier age than in the United States, have *not* died out in this limited, semi-isolated population. Possibly the convalescent carrier stage of these infections accounts

for their persistence on Guam. This I doubt, however, for the carrier states are usually relatively brief, although a very few may remain carriers of diphtheria and streptococci for many months. If we presume that acquired immunity resulting from infection prevents reinfection and is permanent as in measles, these infections should certainly have to die out on Guam, until reintroduced.

Immunity to diphtheria has been studied intensively for many years, yet knowledge regarding the duration of naturally acquired active immunity and the effect of this immunity on the development of the carrier state is still poorly understood. It is generally accepted for diphtheria that many Schick negative (immune) persons *can* become reinfected and so serve as carriers. Both antitoxic and antibacterial immunity are probably involved; the latter very inadequately studied. The most apparent function of immunity in this infection is protection against clinical manifestations, principally due to the toxin. Most healthy carriers have antitoxic immunity. Duration of this naturally acquired immunity without stimulation by reinfection is not well defined, but there is at least one record of a large number of children who became Schick negative as a result of infection (not artificial immunization), reverting to the Schick positive state in 4 years (14).

Much less is known about immunity in streptococcal infections and the problem is greatly complicated by type specificity and the many types of demonstrable antibody, but, in general it is conceded that immunity may not prevent reinfection and the carrier state. In all probability, in diphtheria and in streptococcal infection, immunity is quite variable, tends to be temporary and is renewed by reinfection. It is also probable that certain levels of immunity, though adequate to modify disease, are not adequate to prevent infection. By inference, from the example of Guam, let us substitute for the hypothesis of permanent complete immunity for poliomyelitis, as in measles and chicken pox, one which holds instead that poliomyelitis immunity resembles that of diphtheria and streptococcal infections. Are there data which would invalidate or further support this hypothesis?

Age trends in disease may be affected by many factors, including age of susceptibility, age of exposure, and the duration of immunity. Therefore, one source of data in regard to duration of immunity may be the study of age trends. Let us consider briefly what comparisons with measles, diphtheria and streptococcal infection may contribute.

It has been pointed out repeatedly by Frost (15) and Aycock (16) that the modal age of poliomyelitis is generally higher in rural than in urban areas. Collins has pointed out that poliomyelitis occurs earlier in the children of low income families than those of the well to do (17). Doull (18) appears to have first pointed out that poliomyelitis together with diphtheria and scarlet fever occurs at an earlier age in the southern United States than further north, also at an earlier age in the tropics. Others since then have also pointed out this trend (19–22). We have been carrying out statistical studies recently in respect to the North and the South in the United States, examining race, population density and other variables. At their present stage these studies (23) confirm the earlier observations, suggesting that climate *per se* is one of the factors which influences the age at which poliomyelitis infection is acquired.

Numerous students of poliomyelitis from several different parts of the world have recently pointed out that a shift has occurred in the age distribution in their area since the earlier decades of the century. It is pointed out that a much higher proportion of cases now is reported in the age groups over 5 than formerly. This trend has also been apparent in California, on the basis of some of our studies (24). However, not all evidence supports this (25–27). Increased reporting of non-paralytic cases, which are more common above 5 years, and changes in the age composition of the population may accout for all of the changes. But, whatever the variation may have been in the age distribution of poliomyelitis, it is probably true that an earlier modal age indicates more rapid spread of the virus in the community, leading to earlier immunity. If immunity is complete and permanent the susceptibles are rapidly exhausted and the rates will fall rapidly in older children as in measles. If immunity is relatively temporary and the carrier state not prevented, it will be constantly renewed by reinfection, where spread occurs most readily, but may wane and lapse where a number of years occur between exposures. Such conditions of waning immunity in some can be postulated in areas where the greatest number of cases is reported as occurring in late childhood and among adults. Now let us examine which of the other diseases of childhood most closely follows the observed age pattern of poliomyelitis.

Measles has always shown a higher age in rural than in urban areas (16, 28). I can recall no studies made on the relation of economic status to age of measles.

Measles has *not* been shown to occur at an earlier age in the Southern States *nor* in the tropics (18, 23); in fact, with the decreasing density of population in most southern and tropical areas measles has shown the opposite age trend to that of poliomyelitis.

Some of our graduate students have been assigned the project of studying the age trend of measles in certain states since the early part of the century, to see if any change could be noted. Data have been difficult to obtain, but it appears that the only trend that can be noted is towards a greater concentration of cases at 5 or 6 years, the previous mode, probably due to better reporting in school age groups. There is thus no observable change to a younger or to an older age.

For diphtheria there is also good evidence for its earlier attack in urban than in rural areas (18, 28). The Schick test has also added to this evidence (29).

In contrast to measles, diphtheria shows the same trend as poliomyelitis in respect to latitude and climate (18, 30, 31). Despite the decreased density of population in southern states and in the tropics, the age at which observed infection occurs is lower, and the Schick test becomes negative at an earlier age (18, 30, 32). It was pointed out before that this early change of the Schick test had occurred on Guam (13). It should be noted as a further parallel that clinical diphtheria, like clinical poliomyelitis is observed less frequently in the true tropics, despite the more rapid dissemination of the infectious agent.

In respect to changes in the age distribution of diphtheria over a period of many years, little can be learned from American records, because artificial immunization has seriously disturbed the pattern.

Since streptococcal infections may also behave in a similar way to diphtheria and poliomyelitis and since artificial immunization has been practiced to such a

negligible extent, the parallels here may be studied more completely. Urban and rural comparisons of scarlet fever, by reported cases (28) and by Dick tests (33) closely parallel those of poliomyelitis and diphtheria.

Zingher (34) has pointed out as a result of Dick test surveys that there are more susceptibles among children of the well-to-do than among those of the lower income group, another parallel to poliomyelitis (17).

From many sources—Brazil (31, 32), Africa, (35, 36), China (37), the Philippines (30), and the United States (18, 38), it has been observed that in general, in the warmer or tropical climates, Dick tests become negative at an earlier age than in the more northern areas. Despite the fact, then, that clinical streptococcal infection is less common in areas of hot climate, we have indirect evidence that under these conditions there is in general, earlier and more rapid dissemination of streptococcal infection. This evidence is of course based on the validity of interpreting a negative Dick test as an immune response. Such an interpretation is accepted by most workers in this field, but is not accepted by all. Mortality and morbidity data also support this, since the percentage of deaths and cases reported due to scarlet fever under 5 to that over 5, is higher in those warmer areas as where such comparisons have been made (16).

Not having found any analysis of scarlet fever age trends in the northern United States, covering a period of many years, we have recently made a brief study of this.

Connecticut and Massachusetts are the only states where we have found ready access to annual scarlet fever morbidity reported by single years of age up to 10. In Connecticut (39) from 1924 to 1945 there has been no change in the modal age—always a peak at either 6 or 7 years of age. In Massachusetts (40) the modal age is 6 from 1932 through 1940. It was the same in Maryland from 1908 to 1917 (28). Thus, based on one crude method of examination no changing trend is noted. New Jersey (41) reports for cases by five-year age groups have been examined about the census years 1920, 1930 and 1940, employing reported cases for the census year, the year before, and the following year. Using age specific morbidity rates (table 1), and examining the ratio of the rate from 0–4 years to that of all ages above 4, the ratio was found to increase with time from 2.2 in 1920, to 2.5 to 3.5, indicating that reported cases are now more concentrated below 5 years than above. Mortality data (table 2) show no change between 1920 and 1940, the age ratios for the three census periods being 9.9, 9.6 and 10.0 respectively. Mortality records are certainly more accurate than morbidity, but they do not necessarily give the same information. California[3] statistics point to a lower age for reported scarlet fever in recent years. These morbidity statistics fail to conform with the generally held opinion of the pattern of poliomyelitis, but we must recall that conclusions drawn on the basis of such data are founded on a long series of assumptions of a very treacherous nature, and that certain careful investigators have pointed out hidden errors in some of the poliomyelitis data (25–27). It should be recalled also that scarlet fever may not be representative of all group A beta hemolytic streptococcal infections.

[3] Tabular data received through the kindness of Miss Geraldine Edwards of the California State Department of Public Health.

The difficulties encountered in interpreting morbidity and mortality statistics force us to seek evidence of a more direct and satisfactory nature. This it was felt might possibly exist in a serological test. At our request, Dr. Nell Hollinger of the School of Public Health has been kind enough to run tests for antistreptolysin "O" on sera which we collected for poliomyelitis antibody studies.

TABLE 1

Twenty year scarlet fever morbidity trend—New Jersey

	THREE YEAR PERIODS		
	1919–1921	1929–1931	1939–1941
Population*			
0–4 years	338,696	329,668	256,264
5+ years	2,817,204	3,711,666	3,903,901
Mean cases†			
0–4 years	1,277	1,350	1,482
5+ years	4,806	6,048	6,448
Rates per 1000†			
0–4 years	3.8	4.1	5.8
5+ years	1.7	1.6	1.6
Ratio of rates			
0–4:5+ years	2.2	2.5	3.5

* Population of census years, 1920, 1930, and 1940.

† Cases based on 3-year average.

TABLE 2

Twenty year scarlet fever mortality trend—New Jersey

	THREE YEAR PERIODS		
	1919–1921	1929–1931	1939–1941
Population*			
0–4 years	338,696	329,668	256,264
5+ years	2,817,204	3,711,666	3,903,901
Mean deaths†			
0–4 years	73	28.7	5.9
5+ years	61	33.6	9.1
Rates per 100,000			
0–4 years	21.5	8.71	0.23
5+ years	2.2	0.91	0.2
Ratio of rates			
0–4:5+ years	9.9	9.6	10.0

* Population of census years, 1920, 1930, and 1940.

† Deaths based on 3-year average.

The true significance of antistreptolysin is still unknown but in general, "the age of distribution of antistreptolysin is similar to that of naturally acquired diphtherial and scarlatinal antitoxin" (42). It is transmitted from mother to infant and gradually decreases in the infant (43, 44), as occurs also with antitoxin. However, antistreptolysin is not the same as antitoxin (45). The titers tend to

remain at a fairly constant level until a streptococcal infection occurs, then it rises much higher within 18 days and remains elevated for weeks or months (44). It has not been used extensively before for this type of survey work. The technic used in the test is that of Rantz (46). Results available at this time on the first 500 sera of our series to be tested have been analyzed for this preview, even though over a thousand sera remain to be examined. Equal numbers of serum specimens were collected from children at each single year of age, beginning at different age levels in different places, depending on results of earlier small samples tested for poliomyelitis antibody. In order to smooth out irregularities still present because of the relatively small numbers tested so far, the figures given are the result of applying the well known cumulative method of Reed and Muench for determining a 50 per cent endpoint (47). In this instance the age at which 50 per cent of the sera would have antibody has been roughly determined. Using 100 units or above as an arbitrary level of antibody, for division in two groups, the following results were obtained (table 3). In San Diego, California[4], a southern city, 50 per

TABLE 3

Age of development of antistreptolysin "O" in sera of normal children*

	AREA AND ECONOMIC LEVEL						
	San Diego		Bakersfield		Texas	Mexico City	Guam
	Upper	Lower	Upper	Lower	Mixed	Mixed	Mixed
Age in years by which 50% develop antibodies.....	6–7	4–5	6–7	3–4	5–6	3	2
Number of sera tested.....	68	81	109	39	90	76	36

* Antistreptolysin titer of 100 units or more.

cent of the children of the upper income families attained this titer between the ages of 6–7, while in the lower income families it was between 4–5 years. What this "economic" factor is we will not discuss just now, but you will recall that in studies by Collins (17), this was shown to correlate with poliomyelitis age distribution and the same had been revealed by the Dick test (34). In Bakersfield, California[5], over 200 miles further north but in the San Joaquin Valley where there are high summer temperatures, the upper income group paralleled San Diego, the 50 per cent age falling between 6 and 7, while the lower income bracket, representing here both whites and Mexican families in "shanty town", fell between 3 and 4 years, one year below San Diego where white Anglo-Americans only were included. A group from central Texas[6], mixed income bracket, fell

[4] Sera collected by members of the staff of the San Diego City and County Health Department, Dr. Alex S. Lesem, Director.

[5] Sera collected by Dr. W. C. Buss of the Kern County Health Department and by Dr. John Forney, with the assistance of Miss Mary Huston, R. N., Dr. C. I. Mead and Dr. R. L. Forney.

[6] Sera sent by Dr. J. V. Irons, Director of Laboratories, Texas State Health Department.

between 5 and 6 years, a mean of the high and low income groups for the other two southern areas. No series has been completed for the more northern San Francisco Bay area, but from evidence in Rantz's work, it is expected to be definitely higher. In Mexico City[7] a relatively high tropical city, the 50 per cent age is 3 years, and in Guam 2 years. We thus have preliminary evidence of an immunologic or serologic nature that streptococcal infection occurs at a very early age in the tropics, though the disease is seldom observed, and that infection spreads less rapidly in the more northern areas where much more disease is manifest.

The immunologic, clinical age, geographic and socio-economic parallels between poliomyelitis, diphtheria and streptococcal infections thus become even more closely established. We will examine those for poliomyelitis and streptococcal infections more carefully, later, in a single chart.

Up to this point I have attempted to avoid references to experimental laboratory, and serological evidences of immunity in poliomyelitis. Before turning to the laboratory, let us refresh our minds on the proposed hypothesis. Immunity is temporary; its maintenance depends on repeated infections, it may not prevent reinfection, though it probably does modify clinical response; the healthy carrier state is common among "immunes". This is put forth as an alternate to the older hypothesis of permanent immunity against disease and the carrier state, from a single infection. Based on statistical observations and "shoe leather" epidemiology alone, without reference to laboratory tests in so far as poliomyelitis is concerned, this proposed concept appears to explain findings at least as well as the older one. At this stage neither should be rejected completely in favor of the other.

Now let us see if the laboratory can offer some help in these problems of immunity and epidemiology in poliomyelitis. Soon after the monkey was found to be a susceptible experimental animal, neutralization tests were performed with human and monkey serum. Aycock and others employed this tool in epidemiological studies, usually using one monkey per serum sample. This tool was crude and the studies were limited by the cost of monkeys. Yet, conclusions drawn from this work have been supported and confirmed by the later and much more extensive studies using large numbers of mice in each test and the Lansing strain of virus. It was observed in the course of the early studies that the proportion of persons with antibodies increased with age and did so more rapidly in urban than in rural areas (48). Also, that if the maternal blood contained antibodies, serum of the newborn infant also contained antibodies (49). These disappeared by the time the infant reached six months of age. Although clinical disease occurred less frequently in southern states, infection occurred just as frequently (50). In other countries and on relatively isolated islands it was found that blood serum of adults regularly contained antibodies (51). Results of these surveys paralleled in a general way those of history surveys of infection in measles and

[7] Sera collected by Dr. Gerardo Varela, Director, Institute of Hygiene and Tropical Diseases, Mexico City, and arranged for by Dr. Wilbur G. Downs, International Health Division, Rockefeller Foundation.

as mentioned earlier, of negative Schick and Dick tests in diphtheria or streptococcal infection.

Those who performed these neutralization tests for poliomyelitis usually interpreted the results to indicate that specific immunity had resulted from infection. However, others, including very capable investigators, considered these poliomyelitis neutralizing "antibodies" to be entirely nonspecific or due merely to "maturation". These latter opinions grew to a large degree because a correlation between antibodies and clinical infection could not be observed. Convalescent and normal persons both had antibodies and usually of equal titer. Antibodies were found in the blood of people in areas where the clinical disease had *never* been observed. Still more significant appeared to be the fact that with few exceptions results of serologic tests at the onset of illness usually were just exactly like those at any time during or after convalescence. In most instances all serum samples from the infected patient either failed to neutralize or were positive. These observations differed greatly from those in other viral diseases associated with demonstrable serum antibodies. So, results, which seemed almost incompatible with specific response to infection, yet had much to suggest that they were the result of infection, were then interpreted by some to be due to differences in "strains" of virus—one group suggesting that the original "human" strain changed in the laboratory by monkey adaptation, another that fundamental immunologic differences, independent of the series of monkey passages, existed between the strain used in the laboratory and that infecting the patient. In other words this latter group considered that the test as usually performed was like using a laboratory strain of Western equine virus when dealing with cases of Eastern equine or St. Louis encephalitis. Some support for this interpretation was obtained from time to time and within recent years the evidence for it has become very convincing.

Since the very important contribution of Armstrong (52, 53), by using the mouse-adapted strains of virus, the earlier, more limited work has been widely extended and confirmed. Doubts are still expressed regarding some interpretations, but considering all the results, the specificity of the test and its usefulness as an epidemiologic tool have been generally accepted and, in my opinion, rightly so. For years, the use of the Lansing strain was challenged. It was called "atypical" and a strain from which conclusions about poliomyelitis could not be drawn safely. However, when the MEF-1 (54) the Philip (55), the W. W. (56), and the Wallingford (57) strains were isolated and the Yale S-K strain adapted to mice and typed (58), and when all were shown to be immunologically identical to the Lansing strain and all were cotton rat and mouse adaptable, many misgivings lost their reasonable basis. Most workers feel now that the Lansing type viruses do produce clinical poliomyelitis in man in many parts of the world, in addition to producing mild infection and antibody. The Lansing type virus has finally been granted a dignified and honorable position among other poliomyelitis viruses, even though stigmatized by rodent pathogenicity (59).

To trace the past of antibody studies in our laboratory at the Hooper Foundation, let us begin with a report of Lansing neutralization tests on acute and

convalescent phase serum from 23 patients in Washington and California in 1941 and 1942 (60). Prior to these dates, serologic tests on paired serum specimens had been reported in only single cases or smaller groups. Development of antibodies or rises in titer were detected in 10 of our 23 cases. Cautious, but over optimistic conclusions were drawn: "The practical application of the mouse neutralization test as a laboratory diagnostic method is obviously limited . . . . As to the application of this test to population surveys, it seems probable that it will serve in the same way as has the monkey test and possesses no advantages other than those resulting from economy." This report has remained unique and for reasons which will soon become obvious was probably considered by many to have involved some error in technic, but the results had been checked and double checked before they were published. Shortly thereafter, Turner and his co-workers (61) from Baltimore reported a much larger series composed of 64 pairs of acute and convalescent sera. Members of this group titrated the serum more completely and presented a detailed companion paper (62) emphasizing the reliability of the test as they performed it. These investigators did not find significant changes between the titers of acute and convalescent sera. The results subsequently reported by Brown and Francis (63) in 74 cases were similar to Turner's. Meanwhile, we made tests on paired sera in 102 additional cases and once again reported the results (64) and more recently Pait, Kessel and Grossman tested pairs of serum from 70 cases (65). This time our results, and those of Pait, Kessel and Grossman, agreed with those of Turner, Brown and Francis. To us it appeared obvious that, except for part of our original series, the results reflected an immunologic difference between the Lansing strain used in the test and the strain of virus that had invoked the illness. However, in our report we expressed certain misgivings regarding the significance of some of the antiviral activity of certain human and animal sera. By then more information was available to support the multiple strain hypothesis, notably that from Kessel's group (66) and an earlier very complete and convincing review by Aycock (67).

At the same time that our last series of tests of patients' serum was carried out, we reported antibody tests on a relatively large series of monkeys infected in the laboratory with various strains (64). Only those infected with Lansing, the MEF-1 and one other virus from Nebraska (human cord material which could not be adapted to monkeys) produced antibodies protective against the Lansing strain. These results added evidence for the strain specificity of the test in monkeys at least.

Even before this, we were convinced that, if during infections with most types of poliomyelitis virus, antibody changes occurred in man, they would have to be demonstrated by using virus of the same immunologic type as that responsible for the infection. We had, therefore, begun a long term project aimed at clarifying this fundamental and crucial problem. Preliminary results of this, after 3 years of work, have been reported recently (68).

Stools and acute and convalescent phase serum were collected in over 50 cases of paralytic poliomyelitis. Virus was isolated from many of the stools, and as many strains as possible were sufficiently adapted to the monkey by serial passage

to permit performance of a satisfactory neutralization test with monkeys. Antibodies against the homologous strain increased in all but one instance in the small series which was finally completed. Unfortunately, this project was beset by many unforeseen obstacles. Most of the twenty-some strains isolated in the three outbreaks studied never became sufficiently pathogenic for the monkey to be suitable for neutralization tests. Two monkey "famines" occurred and most of the monkeys obtainable on the West Coast, it appears in retrospect, were those

TABLE 4

Monkey neutralization tests with acute and convalescent phase sera from patients, tested against homologous viruses isolated from the same patients

PATIENT AND VIRUS	RESULTS* MONKEY SERUM CONTROLS	PATIENT'S SERA					
		Acute			Convalescent		
		Days after onset	Serum dilution	Results*	Days after onset	Serum dilution	Results*
4	4/4	5	Undil.	0/2	74	Undil.	0/2
			1:5	0/2		1:5	0/2
			1:25	2/2		1:25	0/2
			1:125	—		1:125	2/2
			1:625	—		1:625	2/2
5	3/3	4	1:5	1/2	24	1:5	0/4
			1:25	2/3		1:25	3/4
6	4/4	4	1:3	0/3	80	1:3	0/3
			1:15	3/3		1:15	0/3
			1:75	1/2		1:75	0/3
7	4/4	4	1:3	0/3	45	1:3	0/3
			1:15	2/3		1:15	0/3
			1:75	3/3		1:75	0/3

* Numerator represents number of monkeys developing paralysis and the denominator the number inoculated.

Table adapted from: Hammon, W. McD. and Roberts, E. C.: Serum neutralizing antibodies to the infecting strain of virus in poliomyelitis. Proc. Soc. Exp. Biol. Med., **69**: 256-258, 1948.

which laboratory workers nearer the source had discarded as unfit for use: they were still less fit when they arrived in San Francisco.

Sera from the first three patients were tested as we ordinarily test for antibodies when working with the encephalitis viruses, the standard method used to that time with poliomyelitis virus. The undiluted serum was mixed with 20 per cent monkey cord suspension and sometimes with an ultracentrifuged concentrate of this when the pathogenicity of the strain was low. In such tests serum obtained during both the acute and convalescent phases completely neutralized the virus. We were surprised by these results, so we repeated the tests until there was no more serum. The results were confirmed. All suggestions in the literature were to the

effect that antibody formed slowly in the monkey, and probably in man. Why then, if we had the right immunologic type of virus, did the patient have antibody at the onset of illness?

In the next four cases (table 4) serial 5-fold dilutions of serum were tested. Here it was again observed that each one of the samples obtained during the acute phase contained antibodies, but in 3 of the 4 instances, antibody was present in lower titer than in serum drawn later.

Finally, by this experiment the fact appeared to be established that in man neutralizing antibodies do increase as a result of infection. *We*, at least, were not convinced in respect to this point until completing these tests. The reason all our patients exhibited antibodies so early in the course of the disease still remains to be found. But, in this respect, poliomyelitis is parallel to Western equine and, to a lesser degree, to Japanese B encephalitis as these infections occur in horses and man. In these two other neurotropic virus infections antibody can usually be detected in significant titer in the first serum specimen.

Having heard that Steigman and Sabin had recently made tests on human serum by a method somewhat similar to one used in our series, I asked for an opportunity to see their results and Dr. Sabin was kind enough to send an abstract of a paper which had just been read (69). Out of 9 cases studied, antibodies were demonstrable in the first serum in all but two cases, but a rise in titer was found in all during the acute phase of disease, thus confirming our findings. They also found that many of the strains of virus isolated were unsuited for tests of this type in monkeys, since the paralytic rates which they produced were exceedingly low.

All of this work lends great assistance to the interpretation of the many incomprehensible results previously reported on the development or lack of development of neutralizing antibodies in human serum during the course of infection. It now appears clear that antibodies are at least partially strain-specific or type-specific, usually develop early in the disease or are already present in low or moderate titers at the time of exposure, owing to a previous infection.

Tests made with laboratory strains from other cases as routinely performed in the past cannot be expected to yield comprehensible results. Neither can it be assumed that a virus isolated from another patient in the same outbreak will be satisfactory (70), for several strains may be and probably are active in many epidemics.

Results of these recent monkey neutralization tests help to clarify the reason why a number of capable investigators were so puzzled by their own results and were led to believe that neutralizing antibodies for poliomyelitis viruses were entirely nonspecific and meaningless.

Now that we can safely assume that antibodies to the Lansing virus result from a poliomyelitis infection let us see what antibody tests can contribute to comparing the trends of poliomyelitis infection with those of scarlet fever and diphtheria.

So far the population of no region in the world has been found free from

poliomyelitis antibody, although in some of these areas clinical poliomyelitis has not been observed or has been only rarely observed. Nevertheless in a number of such areas Americans or Europeans have developed the disease with a notable escape of the intimately associated natives. This strongly points to the likelihood of immunity on the part of natives. Gear (71) has reported rarely finding cases of poliomyelitis among natives of tropical Africa, although high rates might occur in whites. In one area completely free from cases, he demonstrated antibodies in the natives, then virus in the feces of a few, proving that infection is active among them even though completely inapparent. Three out of nine children tested by Gear were excreting virus when tests were made! This closely parallels the tropical situation for diphtheria and streptococcal infection.

Sabin (72) has mentioned that he and Young have tested about 300 sera from children of the Far East for antibodies to the Lansing strain and found that antibodies developed earlier there than in Cincinnati and earlier than in a normal group of Baltimore negroes tested by Turner and associates. We collected sera from some of these same Far Eastern areas, also from Guam, Kwajalein, American Samoa, Mexico and several cities in the United States to attempt to test the correlation of age of developing neutralizing antibody with the age of reported infections. Tests on over a thousand of these sera are still incomplete but a prevue of 635 will be offered here (table 5). It appears from these partial results that antibodies develop much earlier in the tropical Pacific Island of Guam (over 50 per cent positive at 1 year of age) than in northern United States particularly San Francisco (50 per cent age in the low income group between 10 and 11), and it also appears that in California antibodies develop earlier as one proceeds south since in Bakersfield and San Diego the upper income group has a 50 per cent age for antibody between 6 and 8 and the lower income groups between 4 and 6. Where reporting of cases occurs this age distribution correlates very closely with the age of the recognized and reported cases. We reviewed these ages recently for 4 California cities (24).

TABLE 5

Age of development of Lansing neutralizing antibodies in sera of normal children*

	AREA AND ECONOMIC LEVEL							
	San Diego		Bakersfield		San Francisco	Texas	Mexico City	Guam
	Upper	Lower	Upper	Lower	Lower	Mixed	Mixed	Mixed
Age in years by which 50% develop antibodies*.....	6–7	5–6	7–8	4–5	10–11	7	2–3	<1
Number of sera tested.....	76	61	92	92	65	84	81	84

* Lansing Neutralizing Antibodies: Neutralization 10 to 100 LD_{50} or more.

In the tropical Pacific Islands where the disease appears to be *very* rare, the antibodies appear *very* early. I believe we may assume that infection in these areas occurs at an extremely early age, and disease, if it occurs at all, is confused

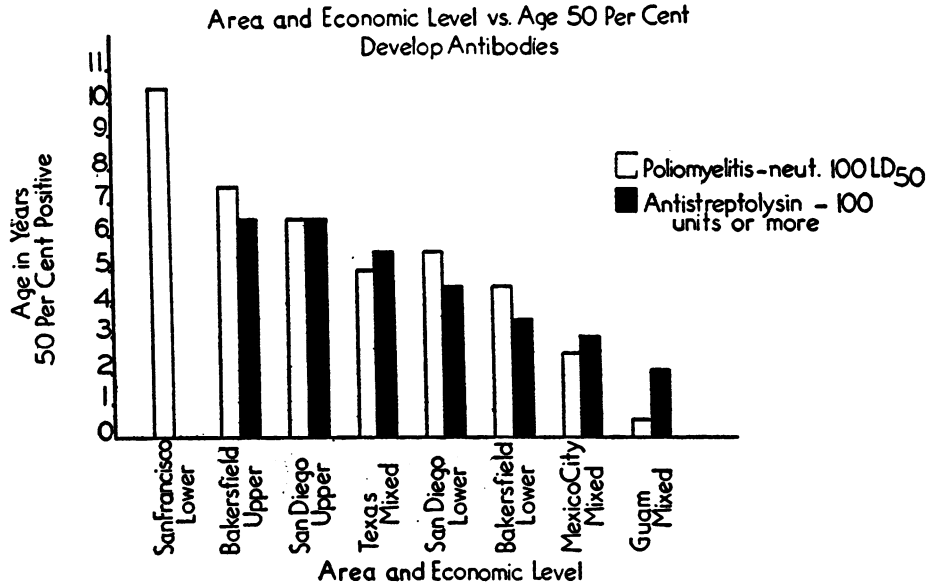
with many other ailments of infancy. Poliomyelitis may even be responsible for a certain amount of the high infant mortality rate. In any case, the association of age of infection where known, and the age of developing antibodies to the Lansing virus appears to be excellent.

Next, let us note the correlation between the age of infection and the economic status of the family. The antibody tests in San Diego and Bakersfield support well the family survey findings reported by Collins (17). Infection develops earlier in poor families. Family size is quite possibly a contributing factor of the economic complex.

Now, let us examine further the correlation of this evidence of an immune response with that of antistreptolysin. Chart 1 presents the results of tests in

CHART I

LANSING NEUTRALIZING ANTIBODIES AND ANTISTREPTOLYSIN "O" IN SERA OF NORMAL CHILDREN



each area and each economic group with antigens of the two infectious agents. The correlation by age, by place and by economic status is most remarkable. If the results continue to bear this relationship when these and additional series on hand have been extended as planned, it would appear highly probable that so many correlations indicate similar epidemiologic patterns; including the effect of immunity on the carrier state and on its duration. It is not intended to infer that the streptococcus and poliomyelitis are etiologically related, in fact this is entirely contrary to our interpretation of the data as will be pointed out in detail in subsequent analysis.

Now let us consider what role neutralizing antibody plays in the immunity of man. I have the temerity to refer again to the arthropod-borne encephalitis

viruses, with which we have had more experience and have developed most of our technics. This is done despite sharp verbal criticisms received in the past from some workers to the effect that the poliomyelitis virus is so entirely different that to suggest parallels with another neurotropic virus is misleading and confusing. This criticism, for the most part, preceded the enlightenment regarding the real significance of neutralizing antibodies and was made at a time when type differences, which have long been recognized in the encephalitides, were not so well established for poliomyelitis. At the moment, it appears quite significant that every one of the immunologic technics, tests, and devices used in encephalitis studies is being employed to advantage in poliomyelitis, including immunization with formalinized or live vaccines, then cross challenge, quantitative serological test of several types, even including complement fixation, and in addition, flocculation (a recent development by Dr. E. C. Roberts in our laboratory). These developments have come in a large part through the work or ideas of several investigators working simultaneously or previously with other types of neurotropic viruses.

For the arthropod-borne viral encephalitides there is considerable reason to believe that neutralizing antibodies play an important role in the immunity mechanism. Here the virus is introduced into the blood or tissue spaces, where it may be inactivated by serum antibodies, probably before it comes in contact with susceptible cells. Furthermore, passive serum immunity to experimentally produced infection by an exact reproduction of the natural route has been demonstrated.

In poliomyelitis, evidence of the role of antibody in immunity is as yet incomplete. It is difficult to be certain that the normal or "natural" route of entry or penetration of poliomyelitis virus has been determined; hence it cannot yet be stated with assurance that infection by the "normal" route has ever been reproduced in laboratory animals. Furthermore, it cannot be assumed with certainty that any laboratory animal is regularly most susceptible to infection through the same portal as man. The erroneous conclusions drawn after dropping virus in the nose of monkeys should remain fresh in our minds. Without knowing the route of invasion we cannot reason logically regarding the barrier that antibody may present to virus. Some, now believe that the infection invoked by feeding virus to chimpanzees serves as a true replica of normal human infection. However, these chimpanzee feeding experiments by Hopkins and Yale workers (73, 74) and similar studies with baby monkeys (75) are the best available at the moment and may serve as the basis for some conclusions about the immunologic mechanism, and, for cautious reasoning, with regard to what may happen in man. After the feeding of any of several types of poliomyelitis virus to chimpanzees (74, 76) and of one type to immature monkeys (77), type-specific neutralizing antibodies developed promptly. After a reasonable interval the virus was shed in the feces for days or weeks, even though the animal showed no signs of illness. In the baby monkey, at least in those in which paralysis accompanied the infection, antibodies were present by the time paralysis occurred (77), although it customarily develops much more slowly in animals inoculated intra-

cerebrally. This same early antibody formation, it should be recalled, was revealed in our tests on normal human paralytic infections (68). The chimpanzees and monkeys, although now endowed with antibody from infection with the original virus strain, when fed a different immunologic type of virus again became carriers. However—and this would appear to be the significant feature—these animals, when fed the same type of virus, within a few months, did not again pass virus in the feces, with only one exception (76), suggesting that they were *immune to reinfection.* At the moment, there seems to be no way to determine whether antibody played any part in preventing infection or interfering with virus fed to these animals in their food. However, in man the antibody found in nasopharyngeal secretions might play such a role.

Amos and Taylor (78) in 1917, and Howitt in 1937 (79) presented evidence for neutralization of poliomyelitis virus by human nasal, mouth and pharyngeal washings, and other studies have also been made on this problem. This neutralizing substance has recently been restudied by Bell (80) and finally identified by him as specific antibody. One may readily speculate on the effect of this antibody found in man, which is so strategically placed in the mouth, throat, and nose, bathing the mucous membranes where the virus may have first contact or may actually penetrate or find receptive cells.

One may also speculate as pointed out by Bell (80) regarding the effect upon the virus which is being discharged from these mucous surfaces during the early stage of infection as such antibody develops during the course of primary infection. Development of antibody may explain the early disappearance of virus from the throat, and thus control this source of infectious material. The fact that virus persists in the throat for only a very short time is well established.

Is there such a mechanism in the intestinal tract at a lower level, or do these antibodies from the pharnyx pass on into the intestines? This has interested us the past several years, under stimulation of the extensive literature on copro-antibody for cholera and bacillary dysentery. The poliomyelitis literature is remarkably silent on this subject. Details of our experiments of the past several years cannot be presented here but a few facts appear to have been established. Extracts of feces of normal monkeys have failed to neutralize Lansing virus, consistently. After establishing this, the MEF-1 strain of virus which is identical immunologically to Lansing virus, was used to inoculate a series of monkeys intracerebrally. This passage material had not been mouse adapted. Extracts of monkey feces collected at 8 and 15 days after paralysis have shown in repeated tests a protective effect of 1.5 to 2 logs against Lansing virus. It appears therefore that some substance with antiviral activity does appear in feces of monkeys inoculated intracerebrally with MEF-1 strain of virus. It remains to demonstrate whether this is, or is not, antibody. It is entirely too early to draw any conclusions about man from these results but it suggests that the feces of man deserve examination.

Morgan has furnished us with some of the best experimental evidence for correlation of immunity and antibody. By giving monkeys a series of intramuscular inoculations of live virus, she has shown a very convincing correlation

between the level of serum antibodies and the resistance to intracerebral inoculation (81), just as she had shown a similar correlation with one of the encephalitis viruses (82). It may be recalled however, that in some of the clinical cases in which we studied the antibody rise by monkey neutralization test with homologous virus, antibodies were readily demonstrable before paralysis, yet the disease progressed to the paralytic state (68). This may simply mean that at that time the antibody titer was not high enough to protect. However, as an indication that other mechanisms may be involved let us note that back in 1936 Sabin and Olitsky showed that convalescent monkeys were resistant to reinfection by the intranasal route before *any* serum antibodies could be demonstrated (83). They also pointed out certain other facts which did not correlate antibody with immunity to experimental disease. Further study of this problem is necessary but at present the possibility of cellular immunity playing a partial role must be strongly considered.

Morgan has recently demonstrated what she believes to be local antibody found in the tissues at the site of poliomyelitis lesions (84). In the intracerebrally inoculated animals these substances appeared before serum antibodies. However, in the light of the much earlier serum antibody formation in monkeys given virus by a more "natural" route (by mouth), by von Magnus and Melnick (77), the significance of Morgan's findings in respect to human infection is very difficult to evaluate.

It now becomes appropriate to consider more carefully the laboratory evidence for the duration, in man, of immunity to reinfection. Reports of several excellent studies (85–89) to detect virus in throat swabs, in feces, or both, made in families where a single case of poliomyelitis is recognized give us extremely interesting and valuable information. Tests for virus on a single specimen of feces or throat secretions collected at some time soon after recognition of the first case in the family, reveal that approximately 80 per cent of the children and 50 per cent of the adults are infected at the time of the single test. If specimens were taken repeatedly and more monkeys employed in the tests there is excellent evidence to indicate that higher proportions would be found infected. Such adult infection rates are extremely rare in measles. On the basis of tests for poliomyelitis antibody with any strain commonly employed, we would expect possibly 50 per cent of the children and 2 to 5 per cent of the adults to be free from antibody. This proportion correlates better with the relative expectation for paralytic poliomyelitis between children and adults, than it does with the observed infection rates (80% and 50%, respectively). So, at first glance, at least, it appears that a high proportion of adults, though possessed with neutralizing antibody and who are presumably "immune", are still susceptible to infection and develop the carrier state. This, to me is the strongest evidence we have of the lack of immunity to infection by those who by every indirect criterion can be assumed to have been previously infected. However, it will be recalled that the chimpanzees and monkeys became immune to reinfection after feeding, for they failed to develop the carrier state when fed the same virus twice. This would appear at first to conflict with the "human" evidence of immunity not preventing reinfection.

But, the laboratory animals were challenged after only a *short* period of time, not after several years as may frequently elapse between re-exposure to the same strain in man. Immunity may be at such a high level for a few months that it ordinarily prevents reinfection, but in its waning phase reinfection is not prevented. I expect this will be proven to be so. At this time it appears probable, in view of known facts, that immunity to reinfection is temporary.

Now, let us consider the duration of acquired neutralizing antibodies, a substance more readily measured. Unfortunately, we can find little evidence to bear on this problem. Man is subject to repeated exposure so continued high titers observed by a few of us in certain individuals mean nothing. I know of no record of repeated tests over a period of years on an isolated, convalescent laboratory monkey. Therefore, at the present, studies on neutralizing antibody do not help us to settle the question of the duration of even this evidence of naturally acquired immunity.

The only other way I can think of to determine duration of neutralizing antibody is to find an isolated island, if such exists, where no neutralizing antibodies to some *one* type of virus are present in the population, and keep there for a number of years a few uninfected individuals who have antibody, or find such an experiment already made to order. Such a situation, where even one immunological type of poliomyelitis virus was absent might answer many questions. Furthermore, the accidental introduction of the missing virus could answer still more questions if competent observers and collection facilities were available. The question regarding adult susceptibility would also undoubtedly be answered. I do not believe that an island lacking infection with one of the several immunologic types of poliomyelitis virus will be impossible to find, once we have a suitable inexpensive serologic test to use for the survey work. Such a test, I am pleased to say may be "just around the corner" on the basis of results which Dr. Roberts of our laboratory is obtaining with a type specific poliomyelitis flocculating antigen (90).

At this point a diversion will be made by way of illustrating an immunity mechanism occurring in another common virus disease. With Dr. John Enders for two years, and later alone, experiments were carried out on a common, world-wide virus disease of cats. Independently and concurrently with Lawrence and Syverton (91), we found this infection had as its chief pathology, destructive lesions of various elements of the hematopoietic system (92, 93). We called the disease panleucopenia. Lawrence and Syverton called it agranulocytosis, and undoubtedly it is the same disease named by others, infectious enteritis, though entirely inaccurately described by that name since there exists no inflammatory lesions. In our immunological studies we found we could passively immunize susceptible kittens by giving convalescent or adult "alley-cat" serum. Vaccination with formalinized cat spleen could also be carried out effectively if the animals were vaccinated several weeks before bringing them to the laboratory, but all susceptible rural cats died after laboratory exposure, unless previously immunized either by serum or by a previous series of vaccine injections (94). It was equally true that city cats and kittens even though the kittens were born in the

laboratory, apparently never became ill. The hypothesis was formed early that the new-born kittens in the city, where infection was apparently omnipresent, were endowed with maternally transferred passive immunity and that they developed a more permanent active immunity through mild or inapparent infection acquired in very early life while still possessing maternal protection (95). This was put to test by routinely inoculating rural susceptible kittens with immune serum once, when introduced into the laboratory. Large numbers of these animals were kept from 6 months to 1½ years and none ever became recognizably ill with panleucopenia (23). The speaker has therefore, long suspected that a similar mechanism might play an important role in immunity to poliomyelitis where conditions were most ideal for its rapid and easy transfer to infants. Some evidence has now been obtained to support this, on Guam at least. Here, all sera tested to date from children between 1 and 4 years of age contained neutralizing antibodies. Dr. Sabin reported that he was unable to demonstrate significant amounts of antibody between 9 months and 23 months in his sera from Japan, Okinawa and Korea and suggested that immunity was not acquired there at such an early age, but further south on Guam the conditions may be somewhat different. This means of safely acquiring immunity, therefore, does not appear to play any important role, except under relatively extreme conditions, probably restricted to certain tropical areas.

No attempt will be made to review in any detail the already extensive literature on the typing of poliomyelitis viruses that has appeared before and since the Virus Typing Committee of the National Foundation for Infantile Paralysis began holding meetings to plan standardized methods and experiments (57, 90, 96–100). By a combination of vaccination and challenge, challenge of convalescents and cross neutralization tests, it appears that at least three distinct immunologic groups of virus exist among the limited number of domestic strains so far tested. Within at least one of these groups there appear to be certain strain differences, revealed most readily by the neutralization and flocculation tests. Already it appears that infection with one can hardly be expected to furnish effective protection against another, any more than St. Louis encephalitis infection can protect against the Western equine type. However, group relationships like St. Louis, Japanese B and West Nile, with some slight antigenic overlapping will probably be found.

The big question in the mind of many is the practical application of all this knowledge to artificial immunity. The speaker's personal opinions and his reasons for them can be presented in a very few sentences.

We will, or we should hesitate a very long time before we use living virus again for poliomyelitis immunization. Inactive virus will produce antibody and temporary immunity to disease in monkeys if given in repeated and very large doses (101). However, a certain danger exists from a type of disseminated encephalomyelitis believed to be due to sensitization with brain tissue components which accompany the virus. Adjuvants added to the monkey cord suspensions have already induced highly severe and fatal lesions in monkeys after just one inoculation (102, 103). Next, killed virus vaccines of any disease result in a very

temporary type of immunity. This could mean that children would require annual vaccination, and once started might have to have it continued for life. To date, only members of the Lansing group of viruses (one immunologic type) can be produced in any animal other than the monkey, and no true poliomyelitis virus has been grown in the chick embryo. Thus, at least two types and possibly many more would have to be made from monkeys, in addition to the Lansing type which could be made from mice. For this the sources of monkeys in the world would be completely inadequate. Furthermore, only one child in hundreds, possibly thousands needs vaccine for protection against crippling, and we have no way to determine which one does. Thus, from the standpoint of danger, necessity of repeated administration, multiplicity of immunologic types, exorbitant cost, insurmountable logistics and difficulty in selection of susceptibles who need vaccination, it all appears to add up to an impractical and seemingly impossible undertaking. Some new development may, of course, completely alter the outlook, but in the meantime I imagine most of our children will be forced to get along with a normal naturally endowed high resistance, supplemented from time to time with a bit of naturally acquired immunity of variable duration and effectiveness. We adults will depend on the same mechanism and all of us may continue to spread virus periodically, at unpredictable times and under unexpected circumstances. On this type of hazardous experience the level of our immunologic status possibly depends, as for streptococcal infections, and until recently, for diphtheria.

It is the speaker's belief that the most practical immediate application of knowledge to be gained in the field of immunity will be in the development of better laboratory procedures—such as serologic tests, and immunologic means of detecting and typing viruses in diagnostic and epidemiological studies.

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